THE ORIGIN AND DEVELOPMENT OF THE INTERNAL MUSCULATURE OF THE FROG LUNG (RANA PIPIENS)

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INTRODUCTION

The general literature regarding the histological structure of the frog lung is quite ambiguous on certain points. There is much disagreement concerning the distribution of the arteries and the veins and their relationships to one another. Whether or not cilia are present in the frog lung is also controversial. Neither is there any agreement about the component parts of the alveolar septa; nor is any information concerning their development available. Smooth muscle is known to be present in the lung, but there is no accurate information concerning its distribution and morphology. Therefore, the writer undertook a histological and embryological investigation of these points.

LITERATURE

The veins and the arteries are distributed as companions to one another, according to the sketches of Renaut (1897), and copied by Oppel (1905), Winterstein (1921), and Holmes (1927). This is in complete disagreement with Küttner (1874), Wiedersheim (1882), and Gaupp (1904), who found the pulmonary artery on the periphery of the lung coursing its way from the root to the apex, giving off numerous lateral branches along the way and a main pulmonary vein on the extreme interior, collecting the blood from the intervening capillary network and returning it toward the root of the lung.

Renaut's diagram of a section through the lung is misleading with respect to the distribution of cilia. He shows cilia present only in the tracheal tube at the root of the lung. On the other hand, Küttner, mentioned above, as well as Hoffman (1878), Haslam (1889), and Königstein (1903), find cilia not only in the tracheal tubes, but also on the margins of the numerous longitudinal and transverse septa on the interior of the lungs.

In regard to the internal septal partitions of the lung, Bourne (1909) has stated that they are found less developed in

the lower portion of the lung wall and are completely absent in the apex. This is contrary to the diagrams of Miller (1893), Renaut, and Oppel, who find them as completely formed in the apex and lower portion as in the region near the root.

Of special interest were the accounts of various writers concerning the smooth musculature of the frog lung. Those will be discussed in chronological order.

Arnold (1863) describes the distribution of the elastic and smooth muscle fibers in the alveolar and septal walls of the frog and of the human lung. He speaks of the smooth muscle fibers as being the essential structural constituent of the frog lung.

Küttner (1874) mentions that the smooth muscle forms in the lung of the frog, "a scaffolding for the support of the arteries and nerves, and for the veins a protective cap located in the crown or inner margin of the numerous septa." He ascribes to this musculature the function of a tension regulator, presumably in reference to the intrapulmonic pressure.

Weidersheim and Haslam state that the lung musculature is arranged in large bands which form a network on the interior from which pass finer processes of muscle tissue in the septal walls toward the periphery where they join a thin and incomplete muscular layer in the external wall of the lung.

Thomson (1899) briefly describes the frog lung as a transparent oval sac with muscle fibers in its walls.

Königstein states that a histological section through the amphibian lung convinces one that the greater part of the compact lung substance consists of smooth muscular tissue. He assigns to this musculature the function of diminishing the size of the lung cavity, so that when the glottis is closed, the air in this cavity will be forced out into the peripherally arranged alveoli.

Gaupp (1904) states that the big veins of the lungs course only on the interior in the crowns (Kuppen) of the septa underneath muscle ridges (Randmuskelbalken).

Oppel (1905) has shown by diagram the distribution of smooth musculature in reference to a lung section and to an individual septal partition. In these his emphasis is entirely upon the distribution of the muscle bundles with complete disregard of the veins which are associated with them, in spite of the fact that he includes blood cells in his sketches. Regarding the blood vessels of the lungs, he relies upon the statements of other authors.

Brown (1909) registered graphically small pressure changes in the lung sac, after inhalation, with the glottis closed and attributed them to the contraction and relaxation of the smooth musculature of the lung.

Carlson and Luckhardt (1920) describe the amphibian lung as a paired muscular sac in which the smooth musculature covers the entire wall and extends into the smallest septa of the interior. The arrangement of this musculature is such that its contraction will reduce the size of the lung cavity and raise the intrapulmonic pressure if the glottis is closed. In their studies they report the discovery of a "persistent peripheral motor automatism" which is normally repressed or controlled by inhibitory impulses from the central nervous system. Between breathing movements, (inhalation and exhalation) when the pause is of sufficient duration, they find an initial rise in the intrapulmonic pressure as did Brown, and, like the latter, conclude that it is due to the contraction of the lung musculature rather than to any other possible external or internal factor.

HISTOLOGICAL STUDIES

1. Methods

Histological preparations were made from fifteen frog lungs (Rana pipiens). Each was obtained immediately after pithing the brain and cord. The lungs were then severed at the roots and at once submerged in Bouin's fixative solution. Some of the lungs were prepared in the collapsed, others, in the inflated state. Each specimen was imbedded in rubberized paraffin for sectioning. Some of the series were stained in Delafield's hematoxylin and eosin; others, in orcein, a specific stain for elastic tissue; still others, in Castroviejo's triple stain, which is particularly good for showing muscular tissue distribution and for showing the presence of erythrocytes in the blood vessels. The blood vessels in most of these lungs were injected with India ink from the heart before being severed from the animal. This procedure very effectively aided the study of the distribution of the blood vessels (arteries, capillaries, and veins) throughout the entire lung structure.

2. Discussion and Results

Arteries

According to Renaut, Oppel, and Winterstein, the arteries and the veins are distributed as companions to one another, whereas Küttner, Weidersheim, and Gaupp found a main pulmonary artery coursing its way from the root to the apex, giving off numerous lateral branches along the way. While a principal artery is present in some cases, this distribution is by no means invariable. Serial sections of four lung preparations show that the pulmonary artery divides into two branches.

One of these again subdivides, forming a total of three vessels of uniform size near the root of the lung.

Cilia

Renaut found cilia to be present only in the tracheal tubes. In agreement with Küttner, Hoffman, Haslam, and Königstein, the writer finds cilia not only in the tracheal tubes but also on the margins of the numerous longitudinal and transverse septa throughout the interior of the lungs.

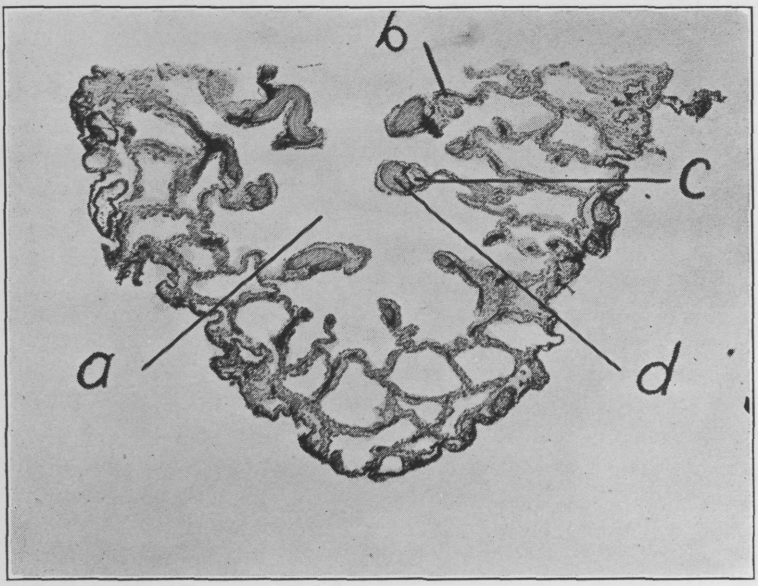

Fig. 1. Longitudinal section near the external wall, showing the peripherally arranged septa: (a) lung cavity, (b) internal septa, (c) small internal vein, (d) internal muscle bundle. 10 ×

Septa

Bourne found the internal septal partitions less developed in the apex of the lung than in the root. This is contrary to the diagrams of Miller, Renaut, and Oppel and to my own observations. They are as completely formed in the apex and lower portion of the lung as in the region near the root. Fig. 1.

Musculature and Venous Return

It is apparent from an examination of the literature that the great amount of disagreement concerns the distribution of the smooth muscle fibers and the venous system of the lungs. A great deal of this is, no doubt, due to the fact that the muscles and veins are structurally so closely associated that many writers investigating the one have failed to observe the other. Because of this close association, it will be necessary to discuss them together.

Most of the authors who have considered the muscular tissue declare it to be a structure independent of the adjacent veins; some of them even failed to observe the closely associated veins. A modification of Castroviejo's triple stain has been particularly helpful in making possible the interpretation of the histological structure of the internal septal marginal veins. Through this stain the muscle tissue appears in green; the white fibrous tissue, in blue; the erythrocytes, in yellow; and the nuclei, in red. With such a stain, that which all the preceding authors have mentioned as an independent muscular network on the interior of the amphibian lung appeared to the writer at first to be a constituent portion of these internal veins. These veins appear to differ from the typical vein in having the smooth muscle tissue, except rarely for a few scattered fibers, assembled asymetrically in a single bundle on their free border. White fibrous connective tissue, which typically is found abundant in both the tunica intima and tunica externa of veins, is, in the case of these septal veins, merged into a single layer for two-thirds of their lumenal circumference. For the remaining third, these layers separate and include the muscle bundle. Moreover, this bundle lies immediately adjacent to the tunica intima of these veins and also possesses numerous interwoven elastic tissue fibers, such as is found in the tunica media of veins. This gives it the appearance of being the tunica media, which has been concentrated in one side.

One cannot escape wondering why there is this peculiar assembling of the muscular tissue of these veins into this single bundle along their internal wall. If the view of the foregoing investigators as to the function of these muscle bundles is accepted, viz., that they act along with the other muscular tissue of the amphibian lung to reduce the size of the lung cavity and thereby raise the intrapulmonic pressure, it is easy to see that from a mechanical standpoint this would be an advantage. A single bundle of muscle fibers located on the free internal border of the septa would act with greater facility and with less impediment in reducing the size of the lung cavity than the same amount of muscle tissue scattered throughout the entire wall of these veins. In a sense this network of muscle bundles is really a complex internal lattice-like sphincter of inter-crossing, longitudinal and transverse, circularly-arranged strands, among which are interspersed elastic connective tissue fibers.

In fact, if the above hypothesis is correct, one may assign two functions to the muscular tissue of these veins, namely: that its contraction will bring about a rise in intrapulmonic pressure and, at the same time, be effective in increasing the blood flow through the pulmonary veins from the lungs. This action would increase the oxygen absorption (Gegenbaur, 1901) of the air in the lung cavity and force the freshly oxygenated blood back to the heart. Both of these functions have their counterpart among mammals in the action of the diaphragm and other inspiratory muscles. Thus, this vascular sphincter would not only perform its normal function of influencing circulation, but also assume a new function of assisting in breathing as well.

Development of the Lung Musculature

The study of the development of the Anuran lung has not extended in the past beyond the very young tadpole. Greil (1905) studied the gross development in *Rana temporaria*, *Bufo vulgaris*, and *Bombinator igneus*. His plates show wax reconstructions of the trachea and lung-bud anlagen, with the veins in blue and the arteries in red. The largest tadpole used was 8.4 millimeters.

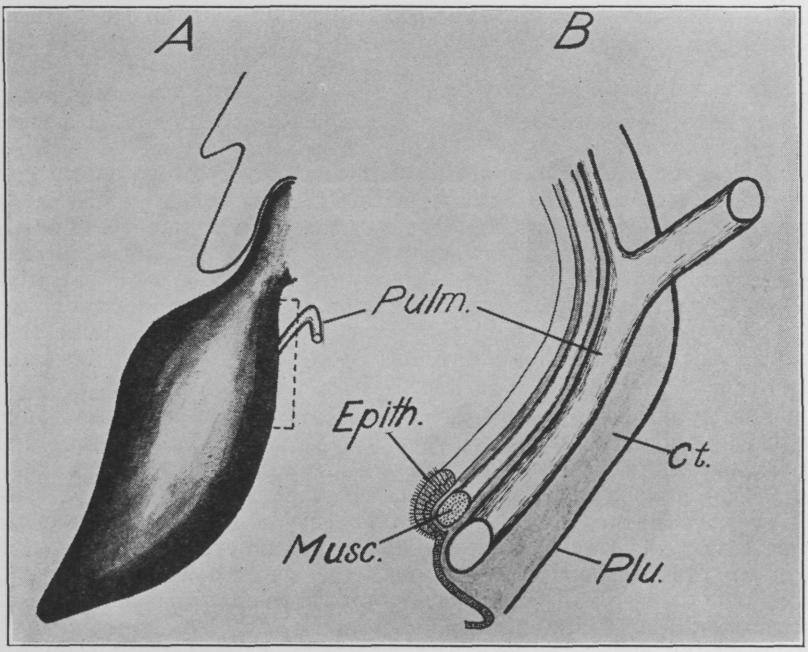

Fig. 2. A. Drawing of the left lung, showing exit of the pulmonary vein. B. Diagram of the dissected lung at portion indicated by dotted line in A. Pulm., pulmonary vein; Epith., epithelium; Musc., muscle; Plu., pleura; Ct., connective tissue.

Fedrow (1910) made a comparative study of the development of the lung veins in various vertebrates. The forms and sizes used were as follows:

Rana temporaria	4.4–11.7 mm.
Triton taeniatus	8.7–11.0 mm.
Lacerta agilis	32–38 somites
Gallus domesticus	3 days
Cavia cobaya	21 days

His object was to show in cross section the similarities of the location and structure of the developing lung sacs and their accompanying blood vessels. In all of his stages, the lung buds were only mere sacs of endoderm with no indication of developing internal septa and smooth muscles.

It seems illogical to think that the muscle network is tunica media because a vein of such construction is, to the writer's best knowledge, not found anywhere else in the animal kingdom. It would seem that the musculature must arise independently of the veins, and the latter become very closely associated with it so as to give a false appearance of being tunica media.

This is borne out by the following facts: The main pulmonary vein originates at the apex of the interior of the lung and courses anteriorly along its meso-lateral border for about three-fourths of the length of the lung. It then passes through the lung wall and proceeds onward to the left auricle. When this vein is on the inside of the lung, it has the same structure as the other veins associated with the musculature. If this muscle bundle is the tunica media of the vein, serial sections of the lung, reading from the posterior to the anterior, should show a transition from the asymmetrical to the symmetrical condition at the point where the vein takes its departure toward the heart. This, however, is not the case. When this point is reached, the muscle bundle continues in association with a tributary vein, while the main vein with a tunica adventitia passes on towards the left auricle. Fig. 2.

Since this problem could not be solved through adult anatomical study, the only alternative left was to study the frog lung from an embryological point of view.

Materials and Methods

The eggs of *Rana pipiens* were collected early in April, and at the time of collection, they were in the neural groove stage. Samples were taken each twenty-four hours for the first week. For the next two weeks, the samples were taken every forty-eight hours and from then on, twice a week until the animals emerged. When the tadpoles reached the stage where hind legs were beginning to develop, the lung buds were carefully dissected out, fixed, and sectioned. The slides were next stained by a dioxan modification of Castroviejo's (1932) method, Waterman (1937).

The critical stages in the development of the frog lung are as follows:

1. Late operculum..... 10.5 mm.
2. ..... 13.5 mm.
3. ..... 16.0 mm.
4. ..... 30.0 mm.
5. ..... 45.0 mm.
6. ..... 60.0 mm.
7. Four legs and long tail..... 58.0 mm.
8. Four legs..... 56.0 mm.
9. Short tail..... 39.0 mm.
10. Very short tail..... 30.0 mm.
11. Tail practically resorbed..... 26.5 mm.
12. Small mature frog
13. Mature frog, medium size
14. Normal mature frog

The sections showing the development of the lung and its musculature were then drawn.

Discussion

The lung develops as an endodermal evagination of the floor of the pharynx. At the late operculum stage, this evagination consists of a single layer of somewhat cuboidal cells. The laryngo-tracheal groove is surrounded by loose mesodermal cells and a few strands of developing

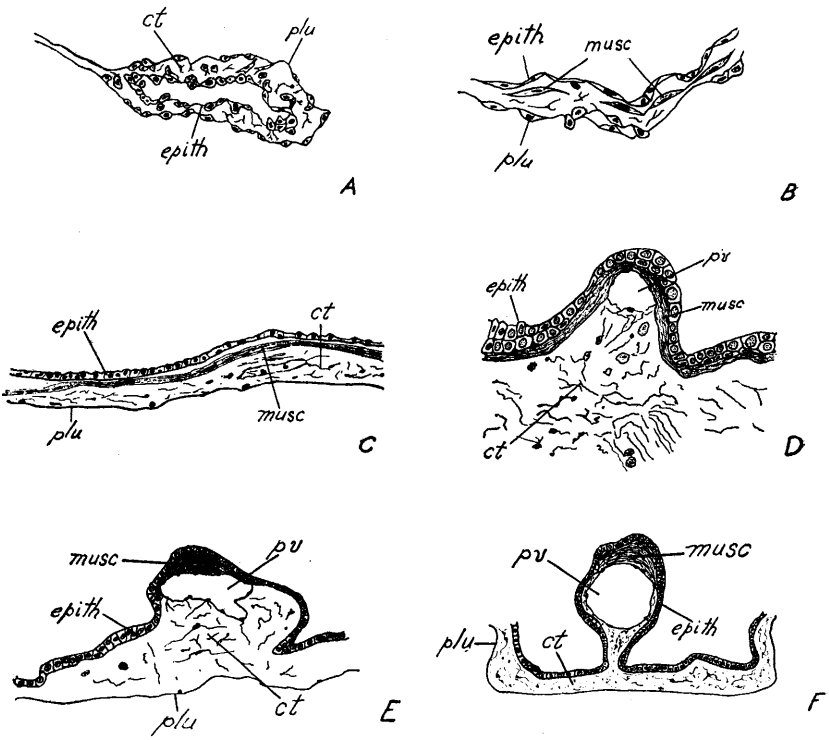

Fig. 3. Camera lucida drawings showing the formation of the alveolar septa and internal musculature of the anuran lung.

A. Lung bud of a 10.5–mm. tadpole of the late operculum stage. epith., epithelium; ct., connective tissue; plu., pleura. 200 ×.

B. Lung bud of a 13.5–mm. tadpole, showing earliest appearances of smooth muscle cells. musc., muscle cells; epith., epithelium; plu., pleura. 400 ×.

C. Cross section of the lung of a 40-mm. tadpole, showing condensed muscle sheet. musc., muscle; plu., pleura; ct., connective tissue; epith., epithelium.

D. Cross section of the lung of a 46–mm. tadpole showing the formation of the rudimentary alveolar septa. pv., pulmonary vein; epith., epithelium; musc., muscle sheet; ct., loose connective tissue. 400 ×.

E. Cross section through the lung of a tadpole with hind legs, showing primitive alveolar septa. Length of tadpole, 56.0–mm. epith., epithelium; musc., muscle fibers; pv., pulmonary vein. 100 ×.

F. Cross section of the lung of a 60–mm. tadpole, taken near the distal end, showing the advanced condition of the musculature. pv., pulmonary vein; musc., muscle bundle; epith., epithelium; ct., loose connective tissue; plu., pleura.

muscle fibers. The anlage of the trachea has the same general structure but more posteriorly becomes somewhat flattened.

The lung buds consist of a single layer of very much flattened and somewhat spindle-shaped cells and are surrounded by very loose connective tissue. No signs of muscle cells are present. Fig. 3A.

By the time the tadpole has reached the length of 13.5 millimeters, muscle cells can be seen developing just under the epithelium. They form a single chain of spindle-shaped cells, which extend around the entire lung. The cells of the epithelium are still flattened, and a few scattered strands of connective tissue can be seen between the pleura and muscle cells. Fig. 3B.

One can best visualize this condition by imagining the lung as being composed of two sacs, exclusive of the plura, one fitting very closely

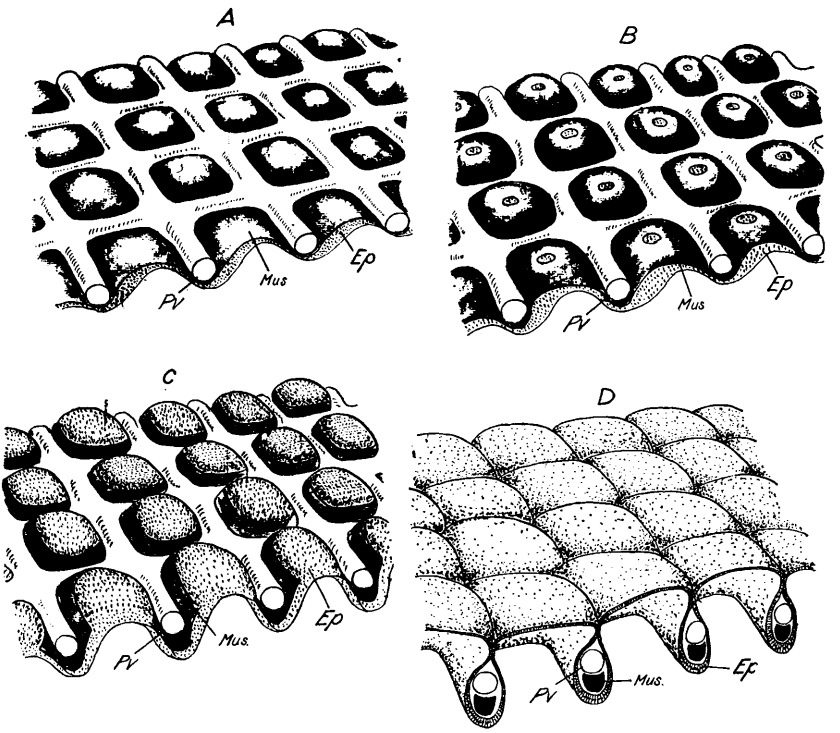

Fig. 4. Diagram showing the formation of the alveolar septa and internal musclature of the Anuran Lung.

A. Lattice-like venous plexus of pulmonary vein with the epithelium and muscular sheets growing through the interstices.

B. Perforations in muscle sheet, exposing the epithelial sheet.

C. Perforations in muscle sheet increase as the muscle thickens over the veins.

D. The muscle is completely condensed over the veins. The epithelium is grown together, forming alveolar septa.

Pv., pulmonary vein; Mus., muscle tissue; Ep., epithelium.

around the other. The inner sac is composed of evaginated endoderm, consisting of very much flattened cells. Around this sac, a second sac fits very closely and is composed of smooth muscle cells, which are loosely joined together.

Very little change can be noticed in the musculature of the lung until the tadpole has reached the length of 40.1 millimeters. At this stage, the muscle fibers have become condensed and extend throughout the interior of the lung just under the epithelium. The cells of the epithelium have become somewhat cuboidal. Fig. 3C.

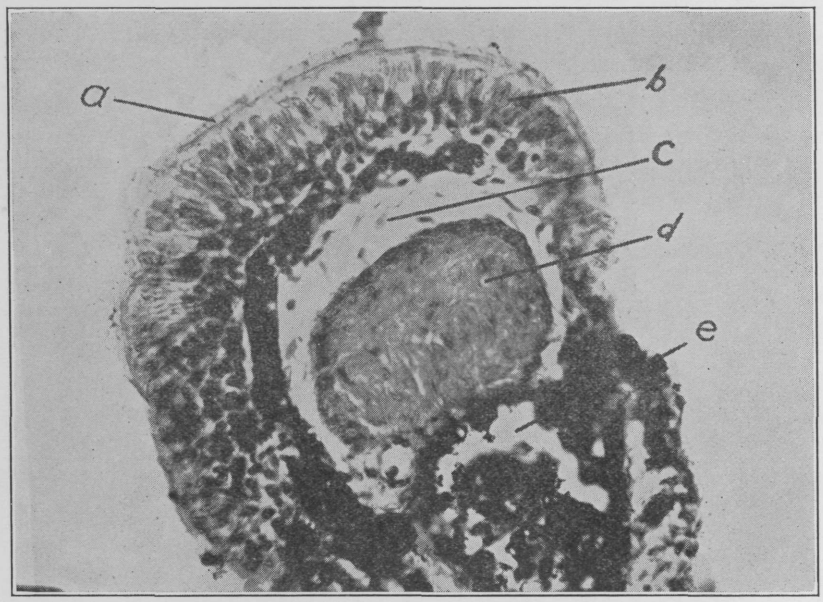

Fig. 5. Highly magnified view of a pulmonary vein and its associated structures: (a) cilia, (b) pseudo-stratified ciliated columnar epithelium, (c) loose white fibrous tissue, (d) muscle bundle, (e) lumen of vein containing India Ink and corpuscles. 800 ×.

The veins can also be seen forming just under the muscle, their walls consisting of a single layer of endothelial cells. The connective tissue underlying the muscle strands is very loose. These veins form a rudimentary lattice-like plexus. The epithelium and muscle strands then grow into the openings of this lattice work. This leaves the veins surmounted by thickening muscle strands and epithelium extending as processes into the lumen of the lung cavity. This marks the first appearance of internal septa. Fig. 3D.

With the appearance of the hind legs, the muscular sac becomes thickened over the internal margins of the venous system. The muscular sac now ceases to be a sac, becomes perforated, and leaves a lattice-like muscular framework that remains permanently associated with the internal border of the venous plexus. Fig. 3E and Fig. 4.

It is interesting to notice that the sections, reading serially from the anterior to the posterior, show that the lungs become more developed toward the apex. This, at first glance, may appear quite contradictory to the well-established fact that an animal develops more rapidly at the anterior end than at the posterior end. However, it is to be remembered that the metabolic gradient, while higher at the anterior end of an organism than the posterior end, is also higher at the ends of the appendages. Thus it would seem logical for the apex of the lung to develop more rapidly than the root, since the lungs arise as appendages of the fore gut, and unless the anterior end remained in a more generalized condition, backward growth would be impossible.

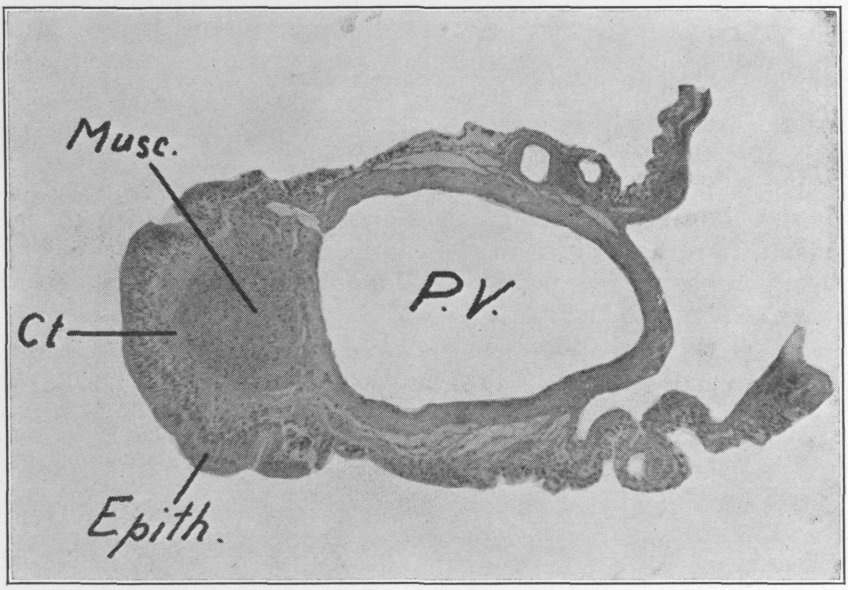

Fig. 6. Cross section through the pulmonary vein of the interior of the frog lung. Musc., muscle bundle; P. V., lumen of pulmonary vein; Ct., connective tissue; Epith., ciliated columnar epithelium.

Towards the apex of the same lung, as shown in Fig. 3E, the musculature, as well as the veins, has become very well developed. The epithelium is now approaching the columnar type, but it is still composed of a single layer of nonciliated cells which are not of the true columnar type as found in the adult organism. Fig. 3F.

Figure 5 shows a high-power photomicrograph of the vein with its connective tissue, muscle bundle, and pseudo-stratified ciliated columnar epithelium. The only comment necessary is that the muscle bundle is not the tunica media of the vein, but it is a structure which has developed in the mesoderm quite independently, and through growth has become very closely associated with it.

The main pulmonary vein, as shown diagrammatically in Fig. 2, when inside of the adult frog lung has the same structure as the other veins. A cross section taken through it shows that the vein and its associated muscles have developed in place and the epithelium has grown around them, leaving a raphae which is visible on the external surface of the lung when examined under high power with reflected light. Fig. 6 shows a photomicrograph through this vein in cross section.

SUMMARY

1. Cilia are found not only in the tracheal sacs at the root of the frog lung, but also on the free internal borders of all the numerous transverse and longitudinal septa.

2. The arteries and veins are not distributed as companions to one another, but independently, the arteries are restricted to the periphery and the veins are located on the extreme interior of the lungs, with the capillaries connecting these relatively widely separated vessels.

3. It is possible for the musculature to affect simultaneously both the intrapulmonic pressure and the venous return of the blood from the lung to the left auricle.

4. Embryonically the frog lung increases in complexity from the root to the apex.

5. The muscle bundle located beneath the epithelium of the alveolar septa is not a part of the tunica media, but is an independent structure, having developed as such, but in close association with the veins.

6. The alveolar septa are formed as follows:

(a) A sac of endodermal epithelium, which has evaginated from the floor of the pharynx, becomes surrounded by a second closely fitting sac, composed of smooth muscle cells.

(b) Around this second sac, a network of blood tubes develops and condenses into a lattice-like plexus of veins.

(c) The epithelial and muscle sheets grow into the openings of this lattice work.

(d) The muscle sheet thins and becomes perforated in the center of the lattice squares and thickens on the lung-cavity side of the veins.

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